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New Observations on the Blood Group Factors in Simiidi and Cercopithecidae*†

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This paper will serve to describe some new findings with regard to the blood group factors in a lowland gorilla (*G. gorilla*), and in monkeys belonging to two species of macaque (*M. mulatta* and *M. irus*).

Since these findings are a direct outgrowth of the discovery that certain conclusions previously reported by one of us (Candela, In press) were incorrect, it may be profitable to recount the successive stages in the investigation in a more or less chronological order.

It has been known for some time that the blood group factors A, B and O, whose presence or absence determine the four blood groups in man, are to be found also in the blood of the anthropoid apes. This fact was first suggested by the finding of group A in one chimpanzee, whose blood was examined by v. Dungern & Hirszfeld (1910-11), and established by the systematic study of Landsteiner & Miller (1925), which revealed that the blood of anthropoids contained also the agglutinogen B, as well as A.

Since that time, the continuation of such investigations of the bloods of Simiidi and Hylobatidae has made it apparent that although all four groups are represented among the apes, each of the genera lacks at least one of the groups[1]. Thus, the chimpanzee, on the strength of about 100 individuals, has only groups O and A, while the orang-utan and the gibbon have only groups A, B and AB. Until recently, the gorilla, represented by only four individuals, was reported to have only group A[2].

This distribution of the blood group factors seemed to imply that the B factor was not present in either of the two African anthropoids. Since the gorilla and the chimpanzee are generally conceded to resemble man more closely than do the Asiatic apes, the apparent lack of the B factor was anomolous, and stimulated various theories postulating its independent origin in the Asiatic apes, and in African man.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

† A more extended report, including details of technique, will be published elsewhere. (Wiener, Candela & Goss, In preparation).

1 This observation applies also to certain of the races of man, the most outstanding examples being the American Indians, the Polynesians and the Australian natives.

2 Actually, as will be pointed out in a later paper (Wiener, Candela & Goss, In preparation), the results quoted on those four gorillas are entirely unreliable. The results reported by Landsteiner (1928) have been misquoted, since this investigator stated that he could not assign a definite group to the single gorilla which he examined. The results reported by Weinert on the remaining three gorillas have been shown by Dahr (1938) to be incorrect, since they are based on an invalid technique.

The importance of these questions to anthropology, and to the problem of human evolution, prompted one of us (C.) to attempt to determine the blood groups of the gorillas in the zoological parks of the United States, by means of tests upon their urines. Such tests are dependent upon the well-known fact that in man, at least, the group-specific substances responsible for the individual blood differences are present in water-soluble form in almost all the tissues and body secretions[3].

The results of the tests on the urines of seven gorillas, three chimpanzees and one orang-utan have been detailed in a previous publication (Candela, 1940). It is sufficient to state that two mountain gorillas (*G. berengei*) were found to belong to group A, three lowland gorillas (*G. gorilla*) to group B, while two other lowland gorillas appeared from the reactions to belong to group AB, and were so reported. Actually, the diagnoses upon these last two were erroneous, for reasons which will presently appear (v.i.).

The dependability of the results obtained by testing ape urine was demonstrated by control tests on twenty human beings, whose groups were determined by independent tests on urine and blood, with complete correspondence. In addition, the blood of the orang-utan, and that of one of the chimpanzees were obtained and tested, and here also the results of the urine tests were confirmed by the reactions of the blood.

Another reason for accepting the urine reactions at their face value lay in the fact that those urines which gave A or B reactions, did so at the same dilution levels found to be suitable for testing human urines. As for the AB reactions, in which the samples removed both the α and the β agglutinins from the test sera, at least one of these reactions, namely that obtained with the urine of the orang-utan, was confirmed by tests upon his blood. It therefore seemed plausible that the two other AB reactions, obtained from the urines of the two gorillas, Janet and Susie, respectively, were equally valid.

Tests upon the Blood of the Gorilla Janet.

An opportunity to check the accuracy of the urine tests on one of these two gorillas came with the transfer of Janet from the New York Zoological Society to the Laboratory of Physiology, The School of Medicine, Yale University, where certain studies were carried out under general anaesthesia.

Blood samples obtained at that time were examined by us, and the unexpected finding was made that the gorilla did not belong to group AB; in fact, it did not correspond exactly to any of the four human groups[4], although it resembled most closely blood of group B.

The serum contained α agglutinins of moderate strength[5], but no β agglutinins, and therefore corresponded with human group B serum. The erythrocytes were tested with anti-A and anti-B testing fluids, prepared from immune rabbit sera. No agglutination at all was obtained with the anti-A serum, and only very weak agglutination with the undiluted anti-B serum, although the latter gave distinct reactions with human B cells in dilutions as high as 1 to 32. Evidently, therefore, the gorilla blood contained an agglutinogen related to, but not identical with the human agglutinogen B. Thus, it is no longer accurate to state that the group factors in the blood of anthropoid apes are in all cases indistinguishable from those found in human blood.

Since the reactions of the gorilla's blood in anti-B serum were weak,

[3] This statement applies only to "secretors." In "non-secretors," who constitute about 20% of white populations, the group-specific substances are absent from the secretions, or present only in traces.

[4] In this respect, our results correspond with those of Landsteiner (footnote 2).

[5] These were accompanied by relatively weak anti-human species agglutinins.

they might have been open to question, in the absence of additional supporting evidence. Accordingly, advantage was taken of the death of the gorilla to study the group-specific substances in its organs. Aqueous extracts of the submaxillary gland were found to inhibit human A (anti-B) serum in relatively high dilutions, but to have no effect on group B (anti-A) sera. This confirmed the grouping of the gorilla's blood as "B-like, α".

The discrepancy between the results obtained from the tests upon the blood, and those previously obtained on the urine of this gorilla, necessitated the re-testing of the urine, not only of this gorilla, but also that of Susie, of the Cincinnati Zoological Society, who likewise had been thought to belong to group AB.

An interesting phenomenon was observed, in that while the undiluted or slightly diluted urines gave AB reactions, when they were diluted 1:6, they both gave B reactions[6], inhibiting the β agglutinin but not the α (Table 1). Since the inhibition of the α (anti-A) agglutinin was thus much weaker than that of the anti-B, the former cannot with any degree of certainty be attributed to the presence of group-specific substance A[7].

TABLE 1.

Results of the re-testing of specimens of urine from Janet and Susie, and three other apes.

Name	Species	Urine Dilution	Residual Agglutinins In: Group A serum	Group B serum
Janet	*G. gorilla*	1:3	—	—
Susie	*G. gorilla*	1:3	—	—
M'bongo	*G. berengei*	1:3	++++	—
Gargantua	*G. gorilla*	1:3	—	++++
Mike	*P. pygmaeus*	1:3	—	—
Janet	v.s.	1:6	—	++++
Susie	"	1:6	—	++++
M'bongo	"	1:6	++++	+++
Gargantua	"	1:6	+++	++++
Mike	"	1:6	++	++

Accordingly, it must be pointed out that the inaccuracy in reporting the groups of Janet and Susie as AB carries no implication as to the correctness of the conclusions arrived at with regard to the remaining nine apes in the series. This is particularly true since eight of these reacted as either group A or group B. With regard to the orang-utan, whose urine gave an AB reaction, his blood also gave an AB reaction, as has been already mentioned.

In summary, it may be said that, (1) The urines of the gorillas Janet and Susie had some adsorptive power for α as well as β, exceeding even the specific reactions of the urines of the remaining nine apes in the series and of the twenty human controls, but when tested at suitable dilution levels, they gave only distinct B reactions. (2) The blood cells obtained from Janet gave very weak reactions in anti-B serum, and none in anti-A serum, while the extract of the submaxillary gland gave a distinct B reaction. The serum of this gorilla contained α agglutinins but no β agglutinins. (3) Based on these new findings, it appears that of the seven gorillas tested to date, all five of the lowland species (*G. gorilla*) belong or are related to group B, while both of the mountain gorillas (*G. berengei*) belong or are related to group A.

[6] But at this dilution, the urines of the other apes did not afford clear reactions, nor did the human urines (Table 1).

[7] The phenomenon of the non-specific adsorption of both the α and the β agglutinins, with the production of false "AB" reactions, has been noted by one of us to occur also in the blood-grouping of human bones (Candela, In press).

Tests Upon the Secretions and Blood of Old-World Monkeys.

The interesting results obtained from the examination of the secretions, blood and tissues of the gorilla Janet suggested the performance of similar investigations upon monkeys.

The available information on the subject of the blood groups of monkeys is based on the examination of the blood alone. The data concerning old-world monkeys can be summarized as follows: (1) The erythrocytes of the Cercopithecidae are not agglutinated by anti-A or anti-B sera. (2) The sera of certain of these monkeys nevertheless contain anti-A or anti-B agglutinins. In the rhesus monkey (*M. mulatta*), anti-A agglutinins but not anti-B are regularly present in the serum; on the other hand, in *Cercopithecus*, anti-B is said to be present, but not anti-A. In baboons and other monkeys, one or the other agglutinin may be present, depending on the individual serum (Landsteiner, 1936; Thomsen & Kemp, 1930; Buchbinder, 1933).

From these observations, it has been concluded that the reciprocal or complementary arrangement of agglutinogens and agglutinins found in man and the anthropoid apes, does not hold true for monkeys. In man, and in the Simiidi and Hylobatidae, when the A factor is present alone in the blood cells, the serum contains agglutinin β, (or anti-B); when only the B factor is present, the serum contains α (anti-A); the absence of both factors from the blood cells is accompanied by the presence of both agglutinins in the serum; finally, when both factors A and B are present in the blood cells, neither iso-agglutinin is found in the serum.

The finding of a definite B factor in the salivary gland of the gorilla Janet, accompanied by distinct anti-A agglutinins in the serum, in the face of the extremely weak B reaction of the blood cells, suggested a new interpretation of the so-called blood group formula. It seemed possible that the lack of reciprocity between the reactions of the blood cells and those of the serum in monkeys might be reconciled by the results obtained from the examination of the tissues and secretions.

The first opportunity to test the validity of this idea arose when the death of a Java macaque (*M. irus*) made available for examination the sublingual gland, together with a block of tongue tissue[8]. The aqueous extract prepared from this material was found to inhibit, in high dilutions, human group A serum, but not to affect human group B serum, thus establishing the presence of group-specific substance B in the tissue extract.

Subsequently, the submaxillary gland and a sample of blood were obtained from a rhesus monkey (*M. mulatta*). The blood serum was found to contain a moderately strong α agglutinin, but the blood cells were not agglutinated by either anti-A or anti-B test sera. The extract prepared from the submaxillary gland inhibited group A serum, but not group B serum, thus yielding a definite B reaction, as anticipated from the presence of the α agglutinin but not the β agglutinin in the serum of the monkey.

In order to determine the presence of group-specific factors in the natural secretions of the macaques, specimens of urine from fifteen monkeys, identified only by numbers, were tested by one of us (C.). Four different kinds of reactions were obtained. In the first four samples, the tests all resulted in group B reactions. Of the remaining eleven, seven gave group A reactions. Three inhibited the A and B test sera equally; these could not be definitely interpreted, nor could a definite conclusion be reached upon the blood group of the monkey whose urine gave only a slight trace of inhibition of the α (anti-A) agglutinin.

The key list obtained after the completion of the tests disclosed the unexpected fact that, although all fifteen specimens of urine derived from macaques, the first four, which had given B reactions, had come from

[8] Except as otherwise indicated, all the material used in this study was personally collected and prepared by one of the authors (G.).

rhesus monkeys (*M. mulatta*), while the remaining eleven, which had yielded seven group A reactions, had been obtained from Java macaques (*M. irus*).

Samples of saliva, obtained from the same fifteen monkeys by means of cotton swabs applied to the floor of the mouth, were tested independently by another of us (W.). These afforded confirmation of the validity of the four B and seven A reactions, and revealed that three of the doubtful individuals belonged to group AB. The fourth, which had yielded an extremely weak A reaction, gave an equally weak A reaction with the saliva. The decision as to group is much easier to make in tests upon the saliva, since this secretion gives specific reactions which are much stronger than those of the urine. For this reason, the failure of the last mentioned specimen (Table 2, number 11), to give a distinct reaction even in tests upon the saliva, suggested the possibility of group O.

The final step in the clarification of the blood group characteristics of this series of monkeys consisted in the testing of their bloods. The results of these tests were in harmony with previous findings obtained with the blood of old-world monkeys, in that the cells could not be agglutinated, while the sera contained group-specific agglutinins.

However, the identification of A-like and B-like factors in the tissues and secretions now made it evident that a reciprocal relation exists in monkeys between group-specific substance in the secretions, and the agglutinins in the serum, just as it does in man and the anthropoid apes between the agglutinogens in the blood cells and the agglutinins in the serum. Every monkey whose tissues or secretions contained the B factor alone, had the α, or anti-A, agglutinin in its blood serum; those in which the A factor alone was found, possessed the β, or anti-B agglutinin in their sera; those three which had both the A and the B factors in their secretions, lacked both iso-agglutinins from their sera, while the one monkey whose secretions suggested group O, had both agglutinins α and β in its serum.

Familial Relationships.

After the completion of the blood group studies upon the monkeys, the family relationships of sixteen of them were clarified by reference to the records of the New York Zoological Society. These sixteen monkeys fall into three families, and their mutual relationships, indicated in Table 2 by the letters x, y and z, may be summarized as follows:

Family x: Java macaques. *Father*: monkey number 00; *mother*: monkey number 7; *children*: monkeys numbers 6 and 15. In this family, the father gave a group B reaction, while the mother and two children reacted as group AB.

Family y: Rhesus monkeys. *Father*: monkey number 2; *mother*: monkey number 1; *children*: monkeys numbers 3 and 4. All members of this family gave group B reactions.

Family z: Java macaques. *Father*: monkey number 5; *mother*: not available; *children*: almost certain, number 8; certain: monkeys numbers 9, 10, 11, 12, 13, and 14. All members of this family gave A reactions, with the exception of number 11, which gave reactions corresponding to group O.

The laws governing the inheritance of the blood group factors in man are, in essence, two: (1) Neither the A nor the B factor appears in an offspring unless it is present in one or the other parent. (2) A parent belonging to group O cannot have a child of group AB, and a parent belonging to group AB cannot have a child of group O.

Although the numbers of monkeys herein reported are too small to permit definite conclusions, it can be said that in these three families of macaques, at least, no exceptions were found to the laws which govern the transmission of blood group factors in humans.

TABLE 2.

Results of tests upon the secretions, tissues and bloods of a gorilla and seventeen macaques.

Name or number and family	Taxonomic name	Source	Reaction from urine*	Reaction from tissues or saliva*	Reaction from erythrocytes*	Group-specific agglutinin in serum	Indicated group
"Janet"	*G. gorilla*	(NYZS)	('AB')				
		(Yale)	(B)	B	B-like	∝	B
(x) 00. Java	*M. irus*	NYZS	not tested	B	not tested	not tested	B
0. Rhesus	*M. mulatta*	R.I.	not tested	B	'O'	∝	B
(y) 1. "	" "	NYZS	B	B	('O')†	(∝)†	B
(y) 2. "	" "	NYZS	B	B	('O')†	(∝)†	B
(y) 3. "	" "	NYZS	B	B	('O')†	(∝)†	B
(y) 4. "	" "	NYZS	B	B	('O')†	(∝)†	B
(z) 5. Java	*M. irus*	NYZS	A	A	'O'	β	A
(x) 6. "	" "	NYZS	O? AB?	AB	'O'	none	AB
(x) 7. "	" "	NYZS	O? AB?	AB	not tested	none	AB
(z) 8. "	" "	NYZS	A	A	'O'	β	A
(z) 9. "	" "	NYZS	A	A	'O'	β	A
(z) 10. "	" "	NYZS	A	A			A
(z) 11. "	" "	NYZS	A? O?	O? A?	'O'	∝, β	O?
(z) 12. "	" "	NYZS	A	A	'O'	β	A
(z) 13. "	" "	NYZS	A	A			A
(z) 14. "	" "	NYZS	A	A	'O'	β	A
(x) 15. "	" "	NYZS	O? AB?	AB	'O'	none	AB

Explanation of Symbols.

x, y, z: Family relationships.

NYZS: New York Zoological Society.

Yale: Laboratory of Physiology, Yale University.

R. I.: Rockefeller Institute for Medical Research.

* : Although the symbols A and B are used to represent the group-specific substances in the tissues and secretions, this is not intended to imply that these substances are completely identical with the corresponding factors in man.

()† : These bloods were not tested, since the findings on the blood of rhesus have been shown to be quite constant.

Summary of Results.

1. A gorilla whose blood group had previously been diagnosed as AB from tests upon the urine, was found actually to belong or be related to group B, as a result of tests upon the blood and tissues. The apparent discrepancy was due to the reactivity of the urine for anti-A as well as anti-B sera, at dilution levels suitable for other specimens; in higher dilutions, the urine gave distinct B reactions. The reactions obtained by tests upon the blood serum and upon an extract of the gorilla's submaxillary gland closely paralleled those of a human group B individual. However, the erythrocytes were only feebly agglutinated by anti-B sera, indicated that the agglutinogen present in the blood was similar to, but not identical with the human B agglutinogen.

Of a total of seven gorillas tested, the revised results reveal that all five of the lowland gorillas (*G. gorilla*) belong or are related to group B, while both of the mountain gorillas (*G. berengei*) belong or are related to group A.

2. Examination of the blood of one rhesus monkey (*M. mulatta*) confirmed the well-known fact that the cells of this primate are not agglutinated by anti-A and anti-B immune testing fluids, while the blood serum contains α (anti-A) agglutinin, but no β (anti-B) agglutinin.

3. The examination of the secretions or the tissues of five rhesus monkeys (including the one mentioned above) elicited the significant fact that a B-like factor, which had been thought to be entirely absent, is actually present, being demonstrable, not in the erythrocytes, but in the tissues and secretions.

4. The Java macaque (*M. irus*) was found to differ in its serology from rhesus, who belong exclusively to group B, in that reactions were obtained corresponding to groups A and AB, as well as group B. In seven of the twelve Java macaques examined the secretions gave a group A reaction, while the serum contained the β or anti-B agglutinin. The eighth individual was found to possess a B-like factor in his tissues; blood from this individual was not available for testing. Of the remaining four, three reacted as AB, in that their secretions inhibited both the α and the β agglutinins, while their sera did not contain either anti-A or anti-B agglutinins. The twelfth Java macaque gave very faint group A reactions from his urine and saliva, but was found to possess both the anti-A and anti-B agglutinins in his blood serum. Although these findings would seem to suggest a diagnosis of group O, further study is necessary for a definite decision.

The finding of an A-like factor in seven Java macaques is believed to be the first demonstration of the presence of the A factor in any monkey.

5. A correlation of the results of the blood group tests with the familial relationships of the three families of macaques discloses no exceptions to the rules which govern the heredity of the blood group factors in man.

Conclusions.

The results of our investigations upon the blood group characteristics of a lowland gorilla, five rhesus monkeys and twelve Java macaques permit certain conclusions to be drawn. These may have new implications for the general problem of the blood groups, and for the questions of the serological interrelationships of the primates.

The findings with respect to the secretions, tissues and blood of the gorilla provide an exception to the rule which holds for the other anthropoid apes, whose bloods gave reactions indistinguishable from those of man.

It is believed that our results have established for the first time the presence, in the tissues and secretions, of both A-like and B-like factors in the two varities of macaques which were studied. These group-specific

factors are in each case accompanied by complementary agglutinins in the serum. Thus, a reciprocal arrangement of agglutinogen and agglutinin previously found only in man and the great apes, is now shown to hold true also in monkeys.

The apparent lack heretofore of such reciprocity was due to the fact that blood group investigations on monkeys have been confined to tests upon their bloods. The reactions obtainable from the secretions and tissues should constitute a valuable implement for future studies in the field of primate blood groups.

There is a suggestion in our results that certain blood group formulae may be distinctive for some of the species of anthropoids and monkeys. The large numbers of studies previously made on the rhesus monkey, reinterpreted in the light of our findings, justify the belief that all members of this species are related to group B. In the instance of the Java macaque, however, even the few herein reported suffice to show that they possess both the A and the B factors. Among gorillas, results from only seven individuals remain available at the present writing, since the four previously reported must now be discounted. These are insufficient to permit deductions; still it may be significant that all five of the lowland gorillas are now known to be similar to group B, and both individuals of the mountain species to group A.

It is significant that the only species of primates in which group O has been found are man and the chimpanzee[9,10]. This suggests the possibility that the O property may have arisen by mutation from either the A or the B factor (probably the former), rather than the reverse mechanism, accepted by some writers, that A and B represent mutations from an original background of group O.

Acknowledgements.

The authors wish to express their gratitude to Dr. John F. Fulton, of the Laboratory of Physiology, of the School of Medicine, Yale University, for his generous co-operation in supplying the blood samples and the salivary glands of the gorilla Janet. We are indebted also to Dr. Karl Landsteiner of the Rockefeller Institute for Medical Research for the blood sample and the salivary gland of a rhesus monkey.

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[9] The O property is not common even in the chimpanzee, since of the one hundred individuals tested, only twelve have been reported to be group O.

[10] Although one of the Java macaques which we tested (number 11) appears to resemble a group O individual, some features of the results indicate caution in the acceptance of such a diagnosis. Further study of this monkey may reveal it to be a non-secretor, with atypical agglutinins (see footnote 3).

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